Amendments to the Specification

Please replace previously presented paragraph [0043] with the following replacement paragraph [0043], first presented marked to show all the changes relative to the previous version of the paragraph and presented second in clean form:

Marked Paragraph [0043]:

[0043] According to the invention, the transmembrane domains of the MRP6 protein shown in FIG. 2 are hydrophobic stretches of amino acids identified via transmembrane domain predictions (SOSUI and DAS transmembrane prediction programs[[~~, http://www.biokemi.su.se/-server/DAS/; http://azusa.proteome.bio.tuat.ac.jp/sosui/~~]]). Regions of MRP6/ABCC6 with a high degree of conservation when compared with similar proteins (ABC transporters) include the regions involved in the binding and hydrolysis of ATP also known as nucleotide binding folds (NBF). According to the invention, the MRP6 protein has two nucleotide-binding fold regions (NBF1 and NBF2) as shown in FIG. 2. These regions correspond to the following amino acid segments of the human MRP6 protein: NBF1 residues 656-679, 747-768, and 775-784 of SEQ ID NO: 3; and NBF2 residues 1292-1307, 1321-1327, and 1403-1433 of SEQ ID NO: 3.

Clean Paragraph [0043]:

[0043] According to the invention, the transmembrane domains of the MRP6 protein shown in FIG. 2 are hydrophobic stretches of amino acids identified via transmembrane domain predictions (SOSUI and DAS transmembrane prediction programs). Regions of MRP6/ABCC6 with a high degree of conservation when compared with similar proteins (ABC transporters) include the regions involved in the binding and hydrolysis of ATP also known as nucleotide binding folds (NBF). According to the invention, the MRP6 protein has two nucleotide-binding fold regions (NBF1 and NBF2) as shown in FIG. 2. These regions correspond to the following amino acid segments of the human MRP6 protein: NBF1 residues 656-679, 747-768, and 775-784 of SEQ ID NO: 3; and NBF2 residues 1292-1307, 1321-1327, and 1403-1433 of SEQ ID NO: 3.

Please replace previously presented paragraph [0055] with the following replacement paragraph [0055], first presented marked to show all the changes relative to the previous version of the paragraph and presented second in clean form:

Marked Paragraph [0055]:

[0055] Intra-familial variation of the phenotype is a well known characteristic of PXE. These variations may be due to genetic and/or environmental causes. A few environmental factors are thought to influence the PXE phenotype. Among these, calcium and Vitamin D have been reported to contribute to the severity of the phenotype in some cases. Life style, smoking, diet, sun-exposure and obesity are also likely to modulate the penetrance of the phenotype. Indeed, remarkably dissimilar PXE phenotypes have been observed recently in identical twins. According to the invention, non-genetic factors contributing to the development of PXE symptoms in heterozygote carriers can be identified. Studies involving large cohorts of twins for example, such as those used by the Queensland Institute of Medical Research of Australia [[~~(http://gene12i.qimr.ed~~]] are also useful to identify both genetic and environmental factors related to the development of the PXE phenotype.

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Please replace previously presented paragraph [0172] with the following replacement paragraph [0172], first presented marked to show all the changes relative to the previous version of the paragraph and presented second in clean form:

Marked Paragraph [0172]:

[0172] Mutation detection, sequence analysis and RT-PCR, SSCP, and Heteroduplex Analysis (HA) were carried out as previously described. Intron-derived primers for PCR amplification of exons present in the genes encoding MRP-1, (MRP6) ABCC6, pM5 and both UNK gene were synthesized using intron sequences available in the TIGR database [[~~(ptti:/Hwww.titrr.org)~~]]. PCR products were typically 150-350bp in length and included complete intron/exon boundaries. Typical PCR reactions, were performed in the presence of .sup.32P-labelled primers in a 9700 thermocycler (Perkin Elmer). Radioactive PCR products were analyzed either by SSCP or HA using MDE polyacrylamide gel (FMC) according to the manufacturer's instructions. DNA conformers were eluted in water from gel slices, re-amplified and sequenced utilizing the same primers used to generate these PCR products. DNA sequence analysis was performed using ABI BigDye terminator cycle sequencing with an ABI310 automated DNA sequencer. The sequence information generated by the sequencer was analyzed using the ABI software. The SEQUENCHER™ [[~~Sequencher.TM.~~]] 3.1 program (Gene Codes Corporation, Ann Arbor, MI) was used to identify variation between the sequence of putative mutations and control sequences. RT-PCR was performed on total RNA from cultured human skin fibroblasts and human kidney poly(A)+ RNA. The sequences of the PCR primers used are: (MRP6) ABCC6: 5'-AGCCACGTTCTGGTGGGTTT-3' (SEQ ID NO: 4); 5'-GGAGGCTTGGGATCACCAAT-3' (SEQ ID NO: 5); MRP-1: 5-CAACTGCATCGTTCTGTTTG-3' (SEQ ID NO: 6); and 5'-ATACTCCTTGAGCCTCTCCA-3' (SEQ ID NO: 7). Following synthesis, PCR products were separated by electrophoresis through 1.2% agarose and visualized by staining with ethidium bromide.

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exons present in the genes encoding MRP-1, (MRP6) ABCC6, pM5 and both UNK gene were synthesized using intron sequences available in the TIGR database. PCR products were typically 150-350bp in length and included complete intron/exon boundaries. Typical PCR reactions, were performed in the presence of .sup.32P-labelled primers in a 9700 thermocycler (Perkin Elmer). Radioactive PCR products were analyzed either by SSCP or HA using MDE polyacrylamide gel (FMC) according to the manufacturer's instructions. DNA conformers were eluted in water from gel slices, re-amplified and sequenced utilizing the same primers used to generate these PCR products. DNA sequence analysis was performed using ABI BigDye terminator cycle sequencing with an ABI310 automated DNA sequencer. The sequence information generated by the sequencer was analyzed using the ABI software. The SEQUENCHER™ 3.1 program (Gene Codes Corporation, Ann Arbor, MI) was used to identify variation between the sequence of putative mutations and control sequences. RT-PCR was performed on total RNA from cultured human skin fibroblasts and human kidney poly(A)+ RNA. The sequences of the PCR primers used are: (MRP6) ABCC6: 5'-AGCCACGTTCTGGTGGGTTT-3' (SEQ ID NO: 4); 5'-GGAGGCTTGGGATCACCAAT-3' (SEQ ID NO: 5); MRP-1: 5-CAACTGCATCGTTCTGTTTG-3' (SEQ ID NO: 6); and 5'-ATACTCCTTGAGCCTCTCCA-3' (SEQ ID NO: 7). Following synthesis, PCR products were separated by electrophoresis through 1.2% agarose and visualized by staining with ethidium bromide.

Please replace previously presented paragraph [0185] with the following replacement paragraph [0185], first presented marked to show all the changes relative to the previous version of the paragraph and presented second in clean form:

Marked Paragraph [0185]:

[0185] Based upon these frequency of heterozygotes and the predicted penetrance of the PXE phenotype in heterozygote carriers (10-20%), heterozygote carriers with PXE symptoms are expected at a frequency of about 0.25% of the general population. In a cohort of about 3000 individuals between 8 and 15 persons presenting cardiovascular, ocular or dermal symptoms would be expected. These numbers provide a basis for a statistical analysis of the correlation between single (MRP6) ABCC6mutations and partial manifestations of PXE. Additional cohorts

with clinically defined cardiovascular abnormalities such as the 1200 sib-pairs group from the Family Blood Pressure Program with hypertension, or the NHLBI Framingham study [[(~~http://www.nhlbi.nih.gov/about/framingham/ -~~)]] from which an appropriate cohort of 2400 to 4500 individuals is available, can be used to provide additional statistical significance.

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